status of the rejections presented in the Office Action, Applicants rely on their belief that claims 1-5 and 7-20 are pending.

Prior to addressing the claim rejections presented in the outstanding Office Action, Applicants believe a brief discussion of the claimed invention, as provided in the accompanying clean copy of the pending claims, would be beneficial.

The invention of the pending claims provides a method of removing bacterial endotoxin from a pharmaceutical process solution that contains an amphiphilic pharmaceutical drug or vaccine substance. The method comprises treating the solution with an ionic surfactant which must be effective to dissociate the endotoxin from the amphiphilic pharmaceutical drug or vaccine substance in the solution. The resulting solution is then filtered through a molecular weight cut-off filter that has a pore size effective to retain the amphiphilic pharmaceutical drug or vaccine substance but allow the dissociated bacterial endotoxin to pass through.

Clean copy of pending claims
1-5 and 7-20

1. A method of removing bacterial endotoxin from a pharmaceutical process solution containing an amphiphilic pharmaceutical drug or vaccine substance which method comprises treating the solution with an ionic surfactant effective to dissociate the endotoxin from the amphiphilic pharmaceutical drug or vaccine substance in the solution, and then filtering the solution through a molecular weight cut-off filter having a pore size effective to retain the amphiphilic pharmaceutical drug or vaccine substance but allow the dissociated bacterial endotoxin to pass therethrough.

2. A method according to claim 1, wherein the pharmaceutical drug or vaccine substance comprises a polypeptide.

3. A method according to claim 2, wherein the amphiphilic pharmaceutical drug or vaccine substance comprises a glycoprotein.

4. A method according to claim 1, wherein the amphiphilic drug or vaccine substance is a vaccine antigen.

5. A method according to claim 4, wherein the antigen is a viral antigen.

7. A method according to claim 5, wherein the antigen is an influenza antigen.

8. A method according to claim 5, wherein the antigen is a haemagglutinin and/or neuraminidase antigen.

9. A method according to claim 1, wherein the surfactant is an anionic surfactant.

10. A method according to claim 9, wherein the anionic surfactant has a steroidal structure.

11. A method according to claim 10, wherein the surfactant is a bile salt or an analogue thereof.

12. A method according to claim 11, wherein the surfactant is a salt selected from the group consisting of salts of deoxycholate, cholate, glycocholate, taurodeoxycholate and taurocholate.

13. A method according to claim 12, wherein the surfactant is deoxycholate (DOC).

14. A method according to claim 1, wherein the surfactant is present at a concentration which is at least as great as the critical micelle concentration of the surfactant.

15. A method according to claim 14, wherein the surfactant is present at a concentration of from one and a half to five times its critical micelle concentration.

16. A method according to claim 15, wherein the surfactant is present at a concentration of between two and four times its critical micelle concentration.

17. A method according to claim 1, wherein the molecular weight cut-off filter comprises a regenerated cellulose acetate membrane, or a polysulfone membrane.

18. A method according to claim 1, wherein, following removal of the bacterial endotoxin, the process solution is subjected to a further process step in which the surfactant is removed.

19. A method according to claim 18, wherein the further process step comprises subjecting the process solution to dialysis.

20. A method according to claim 7, wherein the antigen is a haemagglutinin and/or neuraminidase antigen.

Claim Rejection Under 35 USC §102, second paragraph

Claims 1-5 were rejected as being anticipated by **Shanbrom *et al.*** (EP 0 083 999). Applicants respectfully disagree and traverse the rejection.

Shanbrom describes *inter alia* a method of depyrogenating proteinaceous pharmaceutical products using a non-denaturing amphiphile in which the pyrogenic material (*e.g.*, an endotoxin) is removed by liquid phase separation following treatment with the amphiphile. A close look at **Shanbrom**, however, reveals that it discloses and claims only the use of non-ionic amphiphiles (*see*, 5:2-3 "a preferably *nonionic* amphiphile"; 5:26-34 "A preferred amphiphile ... Nonionic substances of the latter type are available commercially ... under the trademark "Triton X" . . ."; claim 1, 17:11-12 "by weight of a nonionic amphiphile ..."). The reference contains no suggestion of the use of ionic surfactants.

In addition, while microfiltration and ultrafiltration are disclosed by **Shanbrom** as a means of filtering biological and pharmaceutical products (*see, e.g.*, 7:24-26), the reference does not teach the series of steps required by Applicants' claim 1. More particularly, **Shanbrom** does not disclose the dissociation of an endotoxin from the pharmaceutical drug or vaccine substance, followed by the filtration of the solution, whereby the drug or vaccine substance is retained on the upstream side of the filter, while the dissociated bacterial endotoxin passes therethrough.

Example 1 of **Shanbrom** refers to depyrogenation of an albumin solution followed by ultrafiltration using Triton X®-100 to dissociate the endotoxin. The treated solution is then filtered through a Millipore® cassette having a 10,000 molecular weight cut-off (hereinafter, "MWCO"). According to this example, neither the retentate (which contains the albumin) or the permeate (which contains the Triton-X®) contained significant endotoxin levels. The fate of the endotoxin fragments is not specified, and presumably, was not tested for.

Thus, Example 1 does not clearly and unambiguously disclose a method in which the dissociated endotoxin passes through the MWCO membrane. Moreover, experiments carried out by the present inventors suggest that ultrafiltration using the 10kD MWCO membrane would not allow the Triton® surfactant to pass through the membrane as a result of the large detergent micelles that form with this particular surfactant. Thus, the person of skill would not view **Shanbrom** Example 1 as inherently teaching a method in which dissociated endotoxin passes through the filter membrane.

As **Shanbrom** neither teaches the sequence of steps required by Applicants' claim 1, nor the use of ionic surfactants as also required by Applicants' claim 1, the reference does not anticipate the claim. As each of the remaining claims depends directly or indirectly from claim 1, they each contain the limitations of claim 1, and therefore, are also not anticipated. Applicants respectfully request that the rejection under §102 be withdrawn.

Claim Rejection Under 35 USC §103(a)

Each of the pending claims was rejected as being unpatentable over **Shanbrom** in view of **McIntire *et al.*** (*Biochemistry*, 8:4063-66; Applicants' Reference CF) and **Schindler *et al.*** (*Jour. Immun. Methods*, 116:159-165; Applicants' Reference CB), and further in view of **Sweadner *et al.*** (*Applied and Environ. Microbiology*, 34:382-85, Applicants' Reference CD).

As the basis for the rejection, the Office asserts **Shanbrom** as teaching each of the embodiments of Applicants' claims except those of claims 12-13 (wherein the surfactant is deoxycholate) and claim 17 (wherein the MWCO filter is a polysulfone membrane). For these deficiencies, the Office looks to **McIntire** (the surfactant is deoxycholate) and **Schindler** (ultrafiltration with polysulfone). The Office finds the motivation to combine these teachings with **Shanbrom** in **Sweadner**, which is said to suggest that employing such a method would reduce the problem of bacterial contamination. Applicants respectfully traverse the rejection.

As a first matter, Applicants disagree with the Office's analysis of the teachings of McIntire, and assert that while the reference describes the use of deoxycholate linked to a microfiltration membrane to remove endotoxins, the reference does not teach a method of removing bacterial endotoxin from a pharmaceutical drug or vaccine substance. Instead, McIntire is concerned with the study of the reversible inactivation of lipopolysaccharides using sodium deoxycholate. The reference provides no mention of the use of deoxycholate to separate bacterial endotoxin from a pharmaceutical drug or vaccine substance.

The present invention addresses the problem of separating amphiphilic pharmaceutical substances from bacterial endotoxins which are also amphiphilic. A known problem with amphiphilic substances is that they can form strong associations with endotoxins and it is believed that complexes may be formed between endotoxins and amphiphilic substances. Consequently, it is very difficult to separate the two without adversely affecting the amphiphilic drug or vaccine substance. This is particularly true in the case of certain vaccines, such as, for example, influenza vaccine, where the amphiphilic vaccine antigens are assembled into complexes (*e.g.*, rosettes). In the specific case of influenza antigen, it is believed that endotoxin is incorporated into the haemagglutinin/neuraminidase rosettes.

Applicants' solution to this problem, as provided by the pending claims, is to treat the process solution with an ionic surfactant, and preferably, an anionic surfactant, so as to dissociate the endotoxin from the amphiphilic drug or vaccine substance. The resulting solution is then subjected to ultrafiltration such that the larger amphiphilic drug or vaccine complex is retained on the upstream side of the filter, while the smaller, dissociated endotoxin fragments pass through the filter.

It will be appreciated by those of skill that a method such as Applicants' must not lead to deactivation or significant loss of the pharmaceutical drug or vaccine substance, yet must also

effectively remove bacterial endotoxins from the pharmaceutical drug or vaccine substance. McIntire provides no information. that would allow one to conclude that deoxycholate could be used to dissociate endotoxin from a complex formed with an amphiphilic drug or vaccine substance, thereby allowing removal of the endotoxin, while at the same time neither destroying the activity of the drug or vaccine substance, or giving rise to significant loss of drug or vaccine. McIntire is concerned solely with the reversible deactivation of the endotoxin *per se.*

The Office also cites Schindler, stating that the reference teaches a method of removing bacterial endotoxin using ultrafiltration with polysulfone. While it may be true that Schindler describes the removal of bacterial endotoxin using ultrafiltration with polysulfone, the ultrafiltration process set forth in the reference is the exact reverse of Applicants' process.

As set forth above, in the method of the present invention, the endotoxin is dissociated from the amphiphilic pharmaceutical drug or vaccine substance and is then filtered through a filter having a pore size effective to retain the amphiphilic pharmaceutical drug or vaccine substance on the upstream side of the filter, yet allow the dissociated bacterial endotoxin to pass through. In the case of Schindler, the whole point of the disclosed method is to retain microbial products such as endotoxins on the filter, rather than allowing them to pass through. Thus, the Schindler method is in complete contra-distinction from the present invention in that the entire intent and purpose of Schindler is to retain the microbial products on the filter and not allow them to pass through it.

As acknowledged by the Office, Shanbrom does not disclose the dissociation of an endotoxin from an amphiphilic drug or vaccine substance followed by the filtration of the solution whereby the drug or vaccine substance is retained on the filter and the dissociated bacterial endotoxin is passed therethrough. Furthermore, as previously discussed, Shanbrom requires the use of a non-ionic surfactant, while the pending claims require an ionic surfactant.

As has been shown herein, the deficiencies of Shanbrom are not cured by the addition of McIntire and Schindler's teachings, and even with the combination, the present invention is not achieved. McIntire does not disclose dissociating endotoxin from an amphiphilic drug or vaccine substance, and Schindler does not disclose the use of ultrafiltration in a manner that leaves the drug or vaccine substance in the retentate and passes the dissociated endotoxin in the filtrate.

Finally, the Office introduces Sweadner as the motivation for combining the previous references. However, this reference also fails to teach essential features of the present claims; instead, Sweadner describes the use of ultrafiltration to remove endotoxins from solution to reduce bacterial contamination thereof. There is however, nothing in the reference that teaches or even suggests the use of an ionic surfactant to dissociate an endotoxin from an amphiphilic drug or vaccine substance.

The only mixtures of pharmaceutical substances and endotoxins disclosed in Sweadner involve solutions of antibiotics containing endotoxin. As described in the example at page 384, column 1, the antibiotics tested (cephalothin and carbenicillin) are not amphiphilic. Furthermore, in the filtration tests carried out, the antibiotics (*i.e.*, the pharmaceutical substance pass through the filter membrane while the endotoxin is retained on the membrane. Once again, this is in direct contrast to Applicants' method wherein it is the pharmaceutical substance that is retained on the filter while the dissociated endotoxin passes through.

In the sole example wherein Sweadner makes any suggestion of a method wherein it is the endotoxin that passes through the filter, involves a solution comprising a virus and endotoxins (385:bottom of col. 1). In this example (which in fact is purely speculative and was not in fact carried out), the authors state "it might be possible to remove endotoxins from viruses by retaining the viruses on a filter such as PSVP or VWSP and disaggregating and washing

through the endotoxin with a solution of EDTA." This example may be easily distinguished from Applicants' claimed process in that viruses *per se* (unless deactivated) are not a pharmaceutical drug or vaccine substance, and as such, Sweadner does *not* disclose or even suggest, a process for the dissociation of amphiphilic endotoxins from amphiphilic pharmaceutical drugs or vaccine substances.

Sweadner concludes at page 385: "the ground work is laid for the application of molecular filtration to the removal of endotoxin from contaminated solutions of small molecules. For example, all low molecular weight drugs, salts and nutritional compounds should easily pass through a PTCG membrane, leaving contaminating endotoxins behind (*i.e.*, on the membrane)." (emphasis added)

Thus, as with previous cited references, the general teaching of Sweadner is the exact reverse of the present invention. In Sweadner, the purification of pharmaceutical solutions is achieved by allowing the small molecular weight pharmaceutical drug or vaccine substance to pass through the membrane while retaining the endotoxin on the membrane.

Accordingly, it is respectfully submitted that, as with McIntire and Schindler, the teachings of Sweadner also fail to rectify the deficiencies of Shanbrom, and because the reference teaches a process that is in direct contrast to the claimed invention, even if one were motivated to combine the teachings of the secondary references with the teachings of Shanbrom, the present invention would not be achieved.

For all the foregoing reasons, Applicants submit that the claimed invention is clearly distinguished from the prior art and respectfully request that the rejection under §103 be withdrawn.

There being no further issues, the application (including claims 1-5 and 7-20) is believed in condition for allowance and such action is courteously requested. Although no fee is believed due at this time, the Commissioner is authorized to charge any deficiency in fee that may be considered to be due in connection with the filing of this paper to Deposit Account 08-1935.

Respectfully submitted,

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